Wingbeat Frequencies of Some Anopheline Mosquitoes of East Asia (Diptera: Culicidae)

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The wingbeat frequencies were studied of some anopheline mosquitoes including vectors of human malaria and filariasis in East Asia. These frequencies showed low values in newly emerged of both sexes, and rose with increasing age during the first 2 days after emergence. Thereafter, the frequencies were maintained at high constant levels for more than several days. The wingbeat frequency of a mated female was not significantly different from that of a virgin female of the same age, while there was a slight difference between a blood-fed female and a non blood-fed one. There was a correlation between wingbeat frequency and wing length in each sex among the 13 colonies of 8 species. The wingbeat frequencies of mosquitoes in a colony varied widely and the standard errors of the mean frequencies overlapped among species and taxa.

INTRODUCTION

It has long been well known that males of Aedes aegypti are attracted to the female flight sound or to an artificial sound in fundamental frequency (Roth, 1948; Wishart and Riordan, 1959). There have also been a few reports on measurement of the wingbeat frequencies of aedine and culicine mosquitoes (Clements, 1963; Washizuka, 1974). Recently, it has also been confirmed in attraction experiments in the Anopheles gambiae complex (Charlwood and Jones, 1979), Anopheles stephensi, Aedes aegypti, Ae. albopictus and Culex pipiens molestus (Ikeshoji, 1981, 1982, 1985), Cx. tritaeniorhynchus and Cx. tarsalis (Ikeshoji et al., 1985), that the frequency of the female wing sound is the most important factor explaining the attraction mechanism. However, whether or not the wingbeat frequency is influenced by changes in the physiological conditions of anopheline mosquitoes is not known. Ikeshoji et al. (1985) applied this acoustic attraction to mosquito control and succeeded in decreasing the insemination rate of the female population of Cx. tarsalis in the field.

The present study was carried out to measure the wingbeat frequency of certain anopheline mosquitoes including the important vectors of human malaria and filariasis of East Asia under various physiological conditions, i.e., body size, aging, mating, blood-feeding, egg development and oviposition, in order to understand the fundamental quality of this action.

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MATERIALS AND METHODS

The mosquitoes used in the experiment were reared by the method of KANDA (1979). All the materials used in this study were from the laboratory colonies of taxa collected in fields as follows: Five taxa of the Anopheles leucophyrs species group, four of which were the An. balabacensis complex, An. minimus and An. aconitus of Cellia series, An. maculatus of Neocellia species and the An. hyrcanus species (subgenus Anopheles). Four taxa of the An. balabacensis complex were: a taxon from Taiwan (bT) (KANDA et al., 1985) which was An. takasagoensis raised to species status by PEYTON and HARRISON (1980), a taxon from Bangkok (bB) in Thailand and two taxa from Perlis (bI) and Kota belud (bK), Sabah in Malaysia. An. leucophyrs (1S) originated from Sarawak in Malaysia. An. minimus were collected from Ishigaki Island (mI) in Japan and from Kanchanaburi (mK) in Thailand. mI and mK were the same colonies as ISG and KCH-1 used by KANDA et al. (1984), respectively. An. aconitus (ac) was collected from Chonburi in Thailand. An. maculatus were collected from Nakhon Phanom in Thailand, and morphologically classified as two type forms, maculatus s. s. (c-m) and willmori (c-w) type, according to REID (1968). The An. hyrcanus species group used were 3 species from Japan. Two of them were An. sinensis (sn) and An. engarensis (en), the same colonies described by KANDA and OGUMA (1978), and one species was An. sineroides (sr) which was used by KANDA and OGUMA (1976).

Measurements of the wingbeat frequency of mosquitoes were carried out as follows. Adults were mounted at the ventral side of the abdomen on the tip of a small piece of cardboard with nail polish as adhesive under ether-anesthesia. They were placed in a room at 25–26°C for more than 1 hr after awaking from the anesthesia. Flight sounds were recorded with a cassette tape recorder (SANYO, minicassette recorder MR-H 100) at ca. 1 cm distance from a microphone when the mosquitoes started beating their wings. The recorded flight sound was then displayed on an oscilloscope by connecting the earphone outlet to the input of the amplifier and the displayed wave was photographed on oscillograph paper. The wingbeat frequencies of 3 to 28 mosquitoes were determined by measuring the wavelengths on the photogram or the oscilloscope directly, and averaged.

Mating was done between 3-day-old females and the same age males by an artificial mating technique. Part of the mated females were used to measure the wingbeat frequency and the remainder were used to confirm the insemination. Three-day-old females mated the previous day were subjected to blood-feeding and fed human blood to satiety. The wingbeat frequency was measured from the time immediately after blood-feeding till the day after oviposition. To examine the relationship between body size and wingbeat frequency, the wing length from axillary incision to apex was measured. Both right and left wing lengths were measured and averaged.

RESULTS

The wingbeat frequencies of the virgin female and male of An. sineroides, An. minimus and Perlis taxon of the An. balabacensis complex were measured in relation to adult development after emergence and the results are shown in Fig. 1A, B and C. The frequencies of both sexes were low immediately after emergence and rose rapidly during the first 2 days remaining at higher constant levels for more than a week thereafter. This
changing pattern of frequency was observed in *An. sineroides*, *An. minimus* and Perlis taxon of the *An. balabacensis* complex as shown in Fig. 1. The wingbeat frequencies of females at the constant levels were almost equal to those of newly emerged males in *An. sineroides* (Fig. 1A) and *An. minimus* (Fig. 1B).

The influences of mating and blood-feeding on the female frequency were examined with Perlis taxon of the *An. balabacensis* complex. The mean frequencies examined on the day of blood-feeding and the following day were significantly different between the blood-fed female ($\bar{x} \pm S.E.$; 433.1 ± 6.5 Hz and 437.3 ± 4.2 Hz, respectively) and the
non blood-fed one (x ± S.E.; 413.3 ± 6.0 Hz and 418.6 ± 5.4 Hz) (Fig. 2A). However, the frequencies were not different thereafter or on the day following oviposition. On the other hand, the frequency of a mated female was the same as that of a virgin as shown in Fig. 2B.

The respective frequencies and wing lengths of females and males of 13 colonies of 8 anopheline species at 4 or 5 days after emergence were measured and their means are plotted in Fig. 3. In both females (Fig. 3A) and males (Fig. 3B), an inverse correlation was found between wingbeat frequency and wing length. The linear regression lines shown by the dotted lines in both figures are based on the equations $y = -100.2x + 756.9$ ($r^2 = 0.628$) and $y = -109.8x + 1,000.8$ ($r^2 = 0.411$), respectively, whereas, the logarithmic regression lines shown by the solid lines are based on the equations $\log y = -0.811 \log x + \log 1,090$ ($r^2 = 0.449$) and $\log y = -0.512 \log x + \log 1,164$ ($r^2 = 0.354$), respectively.

The mean frequencies of females of the 13 colonies were distributed between 260 and 510 Hz, while those of males were between 510 and 800 Hz. The frequencies of individuals in each species and taxon were variable and the ranges were wide and overlapped. When the mean frequencies of the two sexes in the same species or taxon were compared, the ratios of male to female ranged from 1.3:1 to 1.9:1, while the ratios of the mean wing lengths of male to female were 0.9:1 to 1.0:1.

DISCUSSION

The wingbeat frequency of mosquitoes changed with their age. It was low on the first day of emergence, after which it rose during the first 2 days and reached a plateau in both sexes of the 3 anopheline species studied (Fig. 1A–C). These results are very similar to that of *Aedes aegypti* in Tischner and Schedl (1955). Mature males probably respond and are attracted to the wing sound of newly emerged males because the frequency of the latter’s sound is the same pitch as that of an attractive female sound the second day after emergence, as Clements (1963) also pointed out.

Wingbeat frequency of a blood-fed female on the day of blood-feeding and the following day was different from that of a non blood-fed one (Fig. 2A). But the difference between the mean values of the two frequencies was only 19.8 Hz on the day of blood-feeding and 18.7 Hz on the following day. In general, the wingbeat frequencies are more variable and range more widely (Ikeshoji, 1981; Fig. 3). Further, there was not significant difference between frequencies of the two groups in the latter half period of egg development, or before and after oviposition (Fig. 2A). It is inferred from this that the slight differences have no influence on the male behaviour toward the female. The result in Fig. 2B indicates that mating also had no influence on the wingbeat frequency of female.

Belton and Costello (1979) pointed out that there is a correlation between the wingbeat frequency and the wing length of female among 13 species of 4 genera, but that “the correlation is not perfect even among mosquitoes of the same genus.” In the present study, however, it was found that there is a good correlation between the wingbeat frequency and the wing length in both sexes among anopheline mosquitoes studied (Fig. 3). In particular, the present data fit better to the linear regressions ($r^2 = 0.628$ and 0.411 in female and male, respectively) than to the respective polynomial regressions ($r^2 = 0.499$ and 0.354). Members of the *An. hyrcanus* species group are long winged and
low pitched, while on the contrary, *An. minimus* are short winged and high pitched. Members of the *An. leucophyurus* species group showed high frequency in spite of their relatively long wing length (Fig. 3A). This fact suggests that they produce more power output in flight and that other physiological factors than wing length are concerned in the frequency.

There was a tendency for the range of frequency of the female to be narrower than that of the male. Nevertheless, the ranges of frequency are quite wide and well overlap among species or taxa. The wide variations of frequencies are advantageous in the reproductive strategy of mosquitoes because they provide a greater chance for successful mating (Ikeshoji, 1981).

Wishart and Riordan (1959) reported that the male of *Ae. aegypti* responded to the sinusoidal sound over a wide frequency range. In addition, it is known that the males of some anopheline mosquitoes respond to the attraction sound (Charlwood and Jones, 1979; Ikeshoji, 1981). Hence, there are many questions which must be clarified about the male response to the female wing sound in the process of mating behaviour of the anopheline mosquito.

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